'STATUS PENGAKTIFAN LIMFOSIT DIKALANGAN PENAGIH-PENAGIH DADAH (IVDU) YANG DIJANGKITI HIV DI KELANTAN'

(LYMPHOCYTE ACTIVATION STATUS IN HIV-INFECTED INTRAVENOUS DRUG USERS (IVDU) IN KELANTAN)

Geran Penyelidikan Jangka Pendek USM

FPP 95/039

NO. AKAUN 331/0500/3059

1/2/95 - 31/7/98

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PENYELIDIK-PENYELIDIK

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INTRODUCTION

Infection with HIV results in profound immunologic changes in the infected host. The host immune system becomes dysregulated and profoundly suppressed culminating in a condition known as AIDS. Although this concept is well accepted, the actual immunopathology of the disease is far from established.

Accumulated evidence have shown that the activation or misactivation of the immune system itself may contribute to disease progression (Pantaleo and Fauci, 1994). Thus, higher levels of activated CD8+ T cells have been shown to correlate with poorer clinical status and lower CD4+ T cell count (Giorgi *et al.* 1993). Although measurement of the CD4+ T cell level is the current "gold standard" used as a prognostic marker of HIV-disease, it is highly variable among different individuals or even within the same individual. In fact, we have recently demonstrated that the percentage of CD4+ T cell, a marker suggested to be more stable and less variable than the CD4+ T cell absolute count (Taylor *et al.* 1989) may not necessarily be suitable to be used as a HIV-disease marker for our local situation (Norazmi and Suarn, 1994). These data point to the need for separate studies in populations and risk groups other than white homosexual men to identify and clarify prognostic indicators for the development of AIDS.

Thus, the possible use of other markers such as activated T cell subsets are being sought, aimed to at least increase the predictive value of CD4+ count, if not to replace it. Since IVDU is the most important risk group for the transmission of this disease in Malaysia, the determination of changes in the cellular activation profile in this group may provide valuble information on the immunopathology of the disease.

OBJECTIVE

The aim of this study was to determine any changes in the subsets of CD8+ T cell expressing one or both of the two selected activation markers i.e. CD38 & HLA-DR using 3-colour immuno-flowcytometry.

MATERIALS & METHODS

Subjects:

Intravenous drug users (IVDU) (all women, aged 20-45 years) at Pusat SERENTI, Pengkalan Chepa were grouped into HIV-seropositive and HIV seronegative *(by both ELISA and Western blotting).*

Cellular Activation Markers:

The activation markers, HLA-DR and CD38 on CD8+ T cells which have been proposed to have a predictive value for assessing disease progression (Kestens *et al.* 1992; Levacher *et al.* 1992) were used. Total lymphocyte population and percentage of CD4+ T cells were also determined for comparison. Panel of mouse monoclonal antibody used is shown in Table 1.

Table 1: Panel of mouse monoclonal antibody (Beckton Dickinson).

Panel	FL1 (FITC)	Specificity	FL2(PE)	Specificity	FL3 (PerCP)	Specificity
1	IgG1	Isotype control	IgG1	Isotype control	IgG1	Isotype control
2	CD4	Th subset	CD8	Ts	CD3	Total T
3	CD3	Total T	CD16/56	NK cells	CD19	B cells
4	HLA-DR	MHC II	CD38	Activated cells	CD8	Ts

Sample Preparation:

Immunophenotyping of the cellular marker were carried out using three-colour flow cytometry (FITC, Phycoerythrin, & Peridinium chlorophyll protein). Peripheral blood from the subjects were obtained via venepuncture and collected in sterile tubes containing ethylene diamine tetracetate (EDTA). The specimens collected were processed within 2 hours of drawing. White cell and differential counts were performed on at leasr 5000 and 3,000 cells respectively using a Haematology Counter. High resolution flow cytometric analysis was performed on peripheral blood samples prepared using standardised lysed whole blood methods (Loken *et al.* 1990). Briefly, 100 ul of whole blood samples were placed into a set of polystyrene tubes containing 10 ul of matching combination of murine monoclonal antibodies directly conjugated with FITC, PE and PerCP (Beckton Dickinson). The samples were then incubated in the dark for 15 min at room temperature after which the RBC were lysed in 2 ml of FACS Lysing solution (Beckton Dickinson). The tubes were then centrifuges and the cells were washed once in PBS, pH 7.2, and fixed in 1% formaldehyde and 10,000 cells were analysed in the FACScan using the LYSYS II Software (Beckton Dickinson).

3-colour immuno-flowcytometry

In this study, 3-colour immuno-flowcytometry analysis of HLA-DR, CD8 and CD38 cells was performed. Samples were light scatter gated on lymphocytes. In analysing the fluoresent plots for activation cell markers, a 'logical markers' were set when necessary to provide a more realistic representation of the stained population. Thus, marker settings were adjusted to best differentiate between fairly discrete cell populations.

RESULTS

The result of immuno-flowcytometry analyses is shown in Table 2. Our result show that the percentage level of CD4+ T cells decreased dramatically in HIV+ IVDU compare to HIV- IVDU. In contrast, the percentage level of CD8+ T cells increased in HIV+ IVDU compare to HIV- IVDU. These findings are consistent with the previous observation as reported by many researchers (Fahey *et al.* 1984; Brinchmann *et al.* 1989; Kaslow *et al.* 1990).

Table 2: Expression of the activation markers on CD8+ T cells (mean % + S.D)

SUBSET	HIV -ve (n=10)	HIV +ve (n=13)	Unpaired t test (p<)
CD4	36.2 + 3.4	14.2 + 9.3	0.0001
CD8	23.3. + 6.1	47.3 + 11.9	0.001
CD8+/HLA-DR+/CD38+	12.0 + 5.5	41.7 + 16.6	0.002
CD8+/HLA-DR-/CD38+	27.9 + 2.0	43.5 + 9.1	0.03
CD8+/HLA-DR-/CD38-	54.0 + 28.6	8.7 + 8.3	0.004
CD8+/HLA-DR+/CD38-	6.1 + 8.2	3.3 + 3.8	n.s

With regard to activation status, percentage levels of two subsets, CD8+/HLA-DR+/CD38+ and CD8+/HLA-DR-/CD38+ were found to be higher in HIV+ve IVDU compared to HIV-ve IVDU. In contrast, percentage level of CD8+/HLA-DR-/CD38- subset is lower in HIV+ve IVDU compared to HIV-ve IVDU, whereas percentage level of CD8+/HLA-DR+/CD38- is not significantly different between the two groups.

DISCUSSION

The complexity of HIV disease process reflected in the heterogeneity of the clinical course of individual infected patients, makes monitoring solely by clinical criteria unsatisfactory. There is thus a need for biologic markers to aid in the monitoring of HIV disease progression and the response to therapy. Profound changes of immune cellular activation occur shortly after infection with HIV and persist throughout the disease course.

CD4 lymphocyte subsets, expressed as an absoulute count or as a percentage of total lymphocyte is one of the most important correlates with the disease stage. In addition, CD4 count is also useful in defining patients at risk of developing opportunistic infections and in guiding preventive treatments. However, there are some problems in relying solely on this parameter at the individual level. CD4 count has been shown to be highly variable among different individuals or even within the same individual (Gorter *et al.* 1992, Norazmi & Suarn, 1995). There is considerable overlap in CD4 counts among the clinically defined stages of HIV infection.

Thus, the possible use of other surrogate markers are actively being sought and enumeration of activated T lymphocyte subsets is therefore one obvious choice, since this marker has been shown to correlate with poorer clinical status and lower CD4 count (Giorgi *et al.* 1993). The display of activation markers such as CD38 and HLA-DR increases significantly on CD8 T cells in HIV infection (Levacher *et al.* 1992)

In this study, consistent with many previous studies (Fahey *et al.*, 1984; Brinchmann *et al.* 1989), we demonstrated that CD4 levels were decreased and CD8 levels were increased in HIV seropositive IVDU compared to HIV-seronegative IVDU. Elimination of CD4 cells may be partly due to apotosis initialy triggered by HIV infection (Gougeon and Montagnier, 1993). Our study also showed that certain activation markers on CD8 subsets were increased in

HIV-infected IVDU. Two subsets of CD8 T cells (CD8+/HLA-DR+/CD38+; CD8+/HLA-DR-/CD38+) from HIV-infected IVDU were observe to increased. Gradual progression of (the two activation markers) HLA-DR+CD38-, HLA-DR+CD38+ towards HLA-DR-CD38+ following progression in HIV can be seen, however direct correlation with decline in CD4 absolute count were not found in this study.

CONCLUSIONS

Our study and many others suggested that T cell activation is related to disease progression. The changes in these cellular activation profile may provide additional surrogate markers for HIV-disease progression, in addition to the CD4 count. Further studies include assessment of the expression of those activation markers on the γδ T cell population since this subset has also been shown to be increased in HIV-infection (Norazmi *et al.* 1995; Mohd-Nor *et al.* 1995; Margolick *et al.* 1991).

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